Indigo and its derivatives – from antiquity to cascade reactions

CHEM401 – Chemistry Honours 2020

Literature Review

Submitted by

Oliver Vieri-Pignatelli

Supervisor:

Prof. Paul Keller

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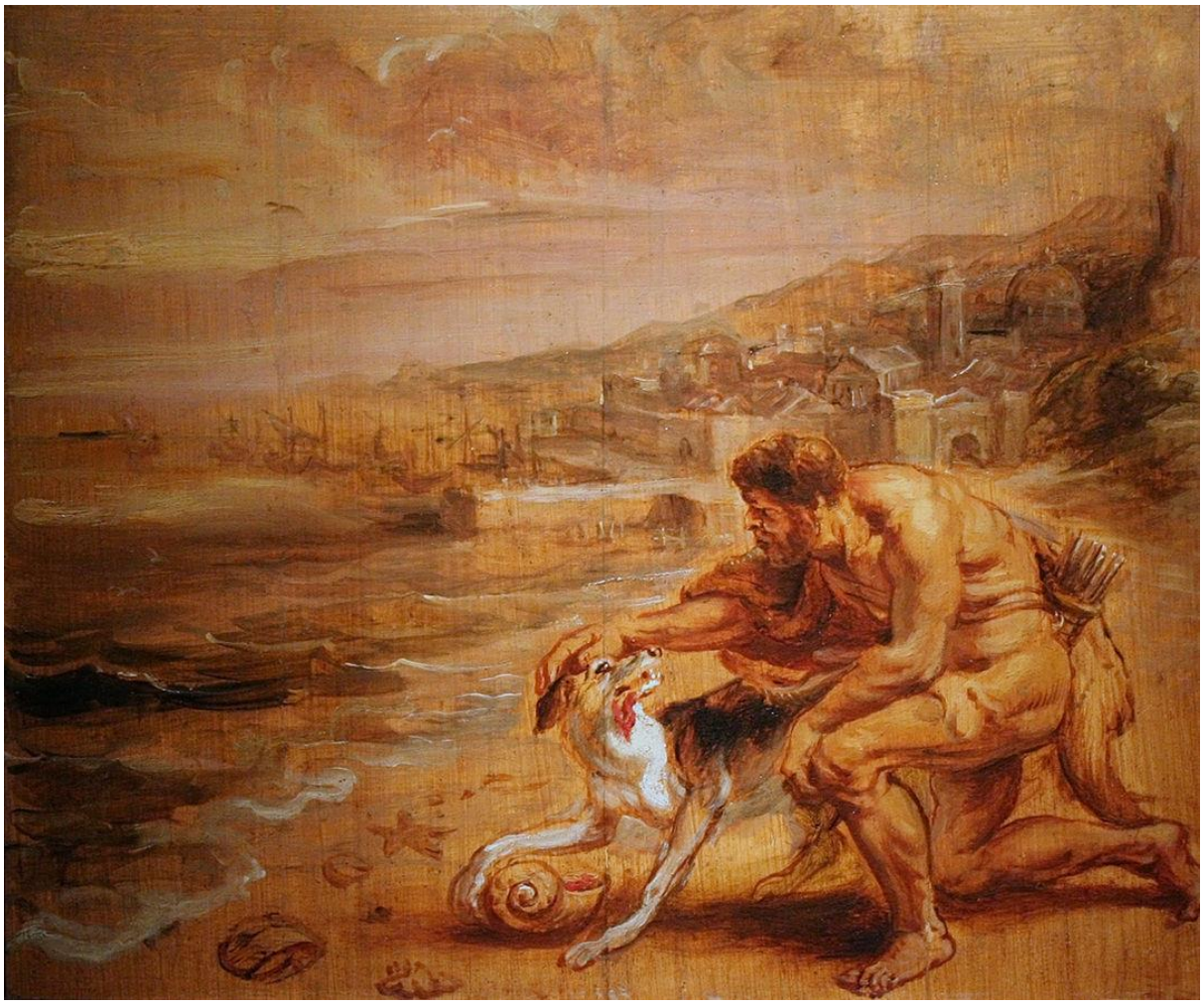

Hercules's and the Discovery of the Tyrian Purple. Musée Bonnat, Bayonne, France. By Flemish artist Peter Paul Rubens (1636).[1] Illustration shows a scene from an origin myth written by second century Greek scholar Julius Pollux depicting the hero Hercules' alleged visit to Phoenicia, where he wooed the nymph Tyro. While walking along the Phoenician shore, his dog bit into a Murex shell and his mouth was stained with purple dye. Seeing this, the nymph Tyro desired a gown of the same colour. The dye became famously known as Tyrian Purple (TP).[2] TP was later identified as an indigoid dye.[3]

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Introduction

Indigo **1** ([2,2′-biindolinylidene]-3,3′-dione) is one of the oldest known natural dyes.[4] It was coveted in ancient times and is a crucial component of the contemporary manufacture of denim.[5,6] The structure of indigo **1** consists of a 2,2′-biindoline core joined by a *trans* alkene bridge, adjacent to secondary anilino groups at positions 1 and 1′ and carbonyl groups at positions 3 and 3′ (Fig 1). Indigo **1** was historically extracted from indigoferous plants and plant-derived indigo **1** is known as indigotin. Indigoferous plants also contain indirubin **2** ((3*Z*)-3-(3-oxo-1,3-dihydro-2*H*-indol-2-ylidene)-1,3-dihydro-2*H*-indol-2-one), which is a structural isomer of indigo **1** (Fig 1).[1,7]

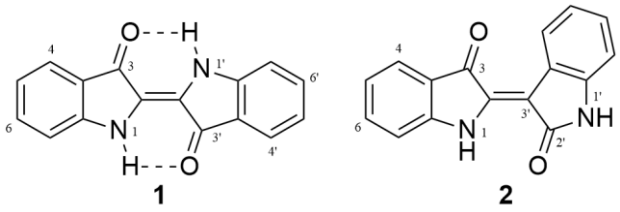


Figure 1: The chemical structures of indigo **1** and indirubin **2**.

The chemistry of indigo **1** has cultural and historical significance. This review explores the history of indigo **1** and highlights its importance in different cultures. Most information about indigo **1** was written pre-nineteenth century and much of the work associated with the chemistry of indigo **1** was performed in the late nineteenth and early twentieth centuries, after the primary source of indigo **1** transitioned from indigoferous plants to synthetic chemistry. A recent revival in interest with indigo **1** has led to the development of cascade reactions and the examination of the medicinal and photophysical properties of cascade products has revealed potential applications for indigo **1** and its derivatives as electroactive semiconducting materials, lead compounds in drug discovery and unique candidates for photophysical research.

History and uses of indigo

Dyeing has been an integral component of human culture since the Neolithic Age (up to 12,000 years before present (YBP)).[8] The first complete description of an indigo **1** dyeing process was reported approximately 2700 YBP.[9] Some of the oldest known textiles dyed with indigo **1** (radiocarbon-dated at 6200 YBP) were found at the Andean Preceramic site of Huaca Prieta, a large stone and earthen ceremonial mound in northern Peru.[1] The indigo **1** from Huaca Prieta was likely extracted from *Indigofera* plants native to South America but indigoid dyes have been extracted from over 700 *Indigofera* species as well as from the *Strobilanthes*, *Isatis* and *Polygonum* genera.[4]

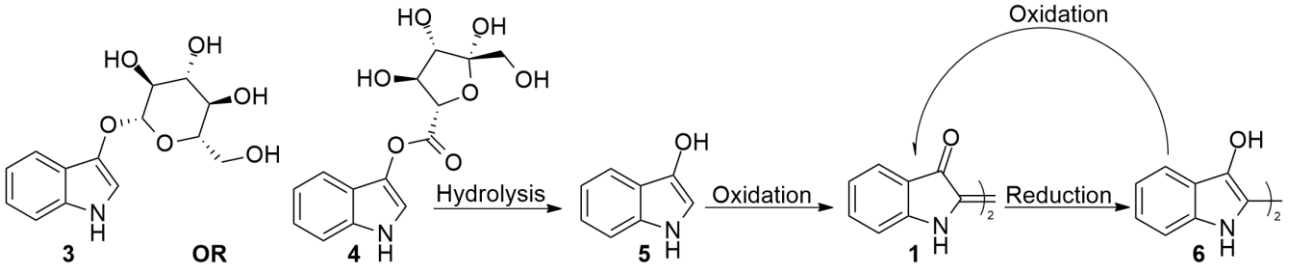
Indigo **1** was known to ancient civilizations in Mesopotamia, Mesoamerica, Iran, Africa and Egypt for millennia.[10] Blue stripes dyed with indigo **1** were identified on an Egyptian mummy aged at

approximately 5000 YBP and the funerary possessions of pharaoh Tutankhamun himself included an indigo-dyed robe (~ 3350 YBP).[11]

India domesticated 'true indigo' (*Indigofera tinctorial*) over 2000 YBP and became the first known base of indigo **1** dye cultivation. India supplied indigo **1** to European countries as early as the period of classical antiquity (2800 – 1400 YBP). The association of indigo **1** with India is reflected in the ancient Greek word *indikón* which means 'from India'.[3,12] The Romans latinised the word *indikón* to *indicum*, which eventually became indigo **1** in English.[10]

In Julius Caesar's firsthand account of the Gallic Wars (approximately 2070 YBP), *De bello Gallico*, his Gaulish enemies were said to have coloured themselves blue with "*vitrum"* (in latin 'glass', which has a similar etymology to 'woad', possibly because both are water-like). The warriors believed the dye to protect and strengthen them and at least it proved intimidating to their enemies. It is now known to have been woad-derived indigo **1**.[4]

True indigo **1** (*Indigofera tinctoria*) cultivated by the Indians contains the precursor indican **3** (indoxyl-β-D-glucoside) whereas the historical European indigo **1** dyeing process involved the preparation and fermentation of the woad plant (*Isatis tinctoria*), which contained the precursor isatan B **4** (indoxyl 3-ketogluconate). The extraction process in either case was similar, starting with the hydrolysis of indican **3** or isatan B **4** to indoxyl **5** and ending with the subsequent oxidation in air to indigo **1.** Using woad resulted in lower quality yields of indigo **1** as opposed to true indigo **1**. A thermophilic, anaerobic *Clostridium* bacterium was employed to reduce insoluble indigo **1** to soluble leucoindigo **6**, which was then mixed with fabric and oxidised in air back to indigo **1** (Scheme 1).[2] This fermentation process has likely not changed for over three thousand years.[13] Stale urine was used by dyers in medieval Europe (1544 – 520 YBP) to reduce indigo **1** to leucoindigo **6**.[12]



Scheme 1: The preparation of indigo **1** from woad (*Isatis tinctoria*) and true indigo **1** (*Indigofera tinctorial*) extracts, starting with isatin B **4** or indican **3**, respectively. Either isatin B **4** or indican **3** was hydrolysed to indoxyl **4** which was subsequently oxidised in air to indigo **1**. Indigo **1** was also reduced in the presence of urine to leucoindigo **6**, mixed with fabric and allowed to oxidise in air back to indigo **1**.

Until the twelfth century in Europe the only source of indigo **1** was India wherefrom it was imported *via* Syria and Alexandria – the transport costs made it expensive.[13] In the thirteenth and

fourteenth centuries true indigo **1** was still rare, and only small amounts were imported through Italy. At this time political tensions surrounded the use of imported true indigo **1**, which was superior in quality to European woad-derived indigo **1**. The 'anti-indigo' lobby succeeded in prohibiting the use of indigo **1** in several countries. Indigo **1** was called the "devil's food" by a religious sect in 1577. The severe anti-indigo **1** restrictions in France were abandoned as late as 1737.[13]

The use of indigo **1** as a dye in Europe seems to have been lost until about 1600 when Dutch mariners reached India and began to trade indigo **1** and with it the knowledge of Indian methods of fermentation. At this point the popularity of indigo **1** began to grow. Approximately 333,500 pounds (~ 150 tonnes) of indigo **1** were imported into Holland in 1631, worth 5 tonnes of gold.[13] By the end of the eighteenth century indigo **1** had become the preferred dye with which the French military leader Napoleon Bonaparte (1769 – 1821) would colour the uniforms of his troops.[4]

Indigo **1** was a staple pigment in early European art despite its rarity and was used in oil paintings by some great painters of the seventeenth and eighteenth centuries including Rubens and Poussin.[4] Blue dyes remained scarce in Europe until 1706 when the paint maker Diesbach in Berlin synthesised Prussian blue **7** ($Fe_7(CN)_{18}$) (Fig 2).[14]

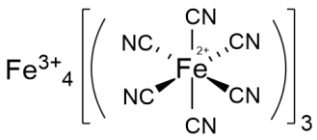


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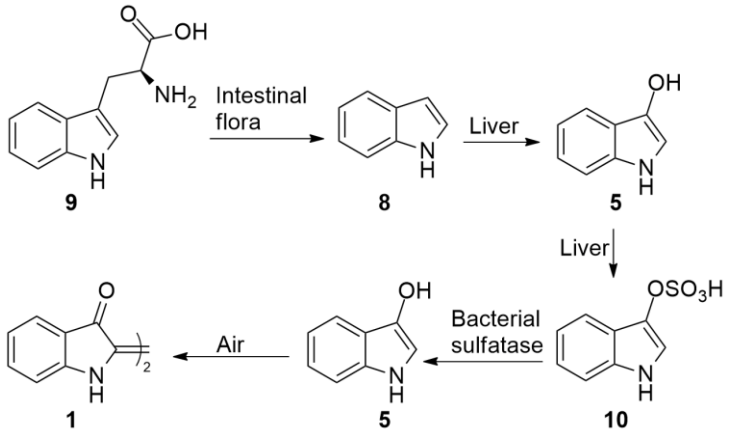
Figure 2: The structure of Prussian blue **7**.

European painters and Japanese painters and woodblock artists, who until the synthesis of Prussian blue **7** did not have access to a long-lasting blue pigment, and had been using smalt (blue glass made by firing in the presence of a cobalt salt), which tended to fade, indigo **1** and even the expensive ultramarine (ground lapis lazuli mined as early as 9000 YBP in Sar-i Sang, Afghanistan).[4,14]

The ancient Mayans (1600 – 1100 YBP) and Aztecs (700 – 500 YBP) employed indigo **1** in their art as a mixture with clay, known today as Maya blue. Despite their age Maya blue pigments still shine brilliantly today due to their extreme stability; they do not fade or change even in the presence of ultraviolet (UV) light and resist boiling, concentrated nitric acid, bases and even organic solvents. The mystery of why Maya blue is preserved so well in a complex with clay is unresolved but is thought to involve hydrogen bonding complexes between indigo **1** and clay.[15]

Indigo **1** is present in certain diseases pathologies. For example, the overproduction of urinary indican **3** was one of many illnesses that plighted the last decade of the life of King George III

(1766 - 1844). Bowel dysfunction led to indole **8** formation from dietary tryptophan **9** by intestinal flora, some of which entered the blood and then the liver where it was converted to indoxyl **5** and then the colourless and water-soluble indoxyl sulfate ester **10**. A bacterial sulfatase was present due to a urinary tract infection and hydrolysis of the sulfate ester **10** saw the slow release of indoxyl **5** which oxidised in air to indigo **1** as seen precipitated on the Royal porcelain (Scheme 2).[16]



Scheme 2: The *in vivo* conversion of dietary tryptophan **9** to indole **8** by intestinal flora, to indoxyl **5** and then the indoxyl sulfate ester **10** by liver enzymes, to indoxyl **5** again by bacteria sulfatase and then oxidation in air to indigo **1**.

The Manchester chemist Edward Schunck (1820 - 1903) spent most of his life investigating natural dyes including indigo **1**; he even coined the term indirubin **2**.[17] While studying indican **3** in 1855 he encountered purple-coloured urine and suggested the colour came from the presence of indigo **1** and indirubin **2**. Schunck devised a test to confirm the presence of indigo **1** in urine: he dissolved evaporated urine in boiling ethanol to precipitate indigo **1** (any indirubin **2** present stayed dissolved). Indigo **1** was then reduced to leucoindigo **6** in the presence of Sn(II) oxide in a basic solution and a blue film of indigo **1** formed on the surface of the solution with exposure to air. Schunck also observed that when indigo **1** was exposed to concentrated sulfuric acid, a dark blue aqueous solution was formed and when heated in a test tube, indigo **1** produced purple vapours which condensed on the walls of the test tube as a blue solid.[18] This is somewhat consistent with Roman naturalist Pliny the Elder's (1997 – 1941 YBP) observation that "to test for indigo **1** the sample should be ignited and indigo **1** identified if a purple flame was seen" (although realistically indigo **1** sublimes at 330 ^{o}C and does not ignite).[4]

Schunck developed a method to detect indigo **1** in urine. He added urine to a basic solution containing Pb(II) acetate, filtered off the white precipitate, and added ammonia to the filtrate, producing yet another white precipitate which he removed and neutralised with dilute sulfuric acid. Schnuck examined the urine of forty healthy working-class people aged between seven and fifty-five using this method and in thirty nine out of forty discovered indigo **1**. Schnuck experimented

with different diets and reported one in particular (large amounts of treacle and arrowroot boiled in water) to be potent in that the following night his urine contained a large amount of indigo **1**.[18]

In the first half of the nineteenth century in India indigo **1** was known as blue gold. It was a huge cash crop due to the demand for blue dye in Europe.[10] The indigo farmers worked under harsh circumstances and were plighted by debt and hardship by European traders. They were even made to plant indigoferous crops in place of food and received little to no compensation. In 1859 in Bengal still worse laws were imposed by the government who favoured the landowners so that the farmers had no choice but to revolt. Although the indigo farmers had the full support of the Bengali middle class, their revolt was ruthlessly suppressed by large forces of police and military, sanctioned by the British government and the landowners. The revolt was clearly illustrated in the report written by the newly appointed "Indigo Commission" in 1860 in which it was written that "not a chest of indigo reached England without being stained with human blood".[19]

Indigo plantation slavery also occurred in North America in the eighteenth century, where it was a major crop, and similar exploitations of local indigenous populations occurred in Jamaica, Haiti, Madagascar and the West Indies, as well as the South American colonies, Guatemala and Mexico, whence Spain imported its indigo **1**.[14] By 1806, 2200 tonnes of indigo **1** had been imported into Great Britain, primarily from India, worth the equivalent of two million pounds. By 1897, India had exported 19,000 tonnes of indigo **1**.[13]

In 1897, 7000 square kilometres of land supported the cultivation of indigoferous plants worldwide[9] but by 1913 this area had decreased to 1210 square kilometers[4] and in 1914 only 4% of the world's indigo **1** production had a vegetal origin.[13] The agricultural industry had given way to synthetic chemistry.

Indigo **1** was first made in 1878 by Adolf Baeyer and Viggo Drewsen and in 1882 they developed the first laboratory scale synthesis.[20] In 1913, following the development of industrial scale syntheses by Karl Heumann and Johannes Pfleger, the companies Ludwigshafen and Hoechst produced a total 8700 tonnes of indigo **1**.[4]

After World War I the Swiss, English, Americans and Japanese began to produce indigo **1** but at first they were unsuccessful because their use of anthraquinone-based vat and sulfur dyes resulted in reduced lightfastness of product, meaning their dyes faded sooner.[4]

Indigo **1** became popular in North America during the vibrant counterculture movement of the 1960s. The otherwise undesirable property of denim (indigo-dyed cotton), its low abrasion strength, was valued by the revolutionaries, who embraced aesthetics rejected by the prim mainstream

culture. The denim industry, which had previously produced 100 tonnes of denim per year, in 1994 produced 14,000 tonnes of denim using industrially manufactured indigo **1**.[4] In 2015 over 50,000 tonnes of indigo **1** was produced industrially.[21]

Physical properties

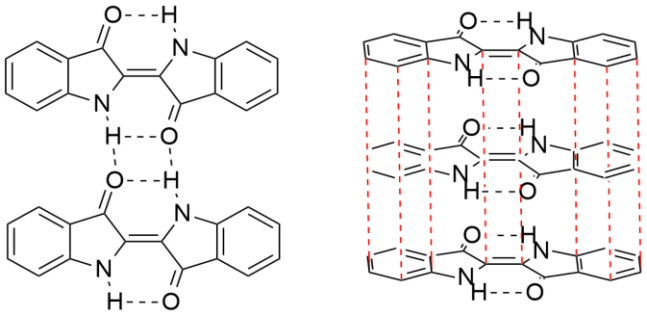


Figure 3: Intermolecular bonds between indigo molecules, showing hydrogen bonding (left) and π -stacking (right) interactions.

Indigo **1** is one of the most stable known organic dyes.[4] The hydrogen bonding that occurs between the adjacent carbonyl and anilino groups (Fig 3) contribute to the poor water solubility, high stability and characteristic blue colour of crystalline indigo **1**. The indigo **1** molecule is symmetrical and belongs to the C_{2h} point group.[20]

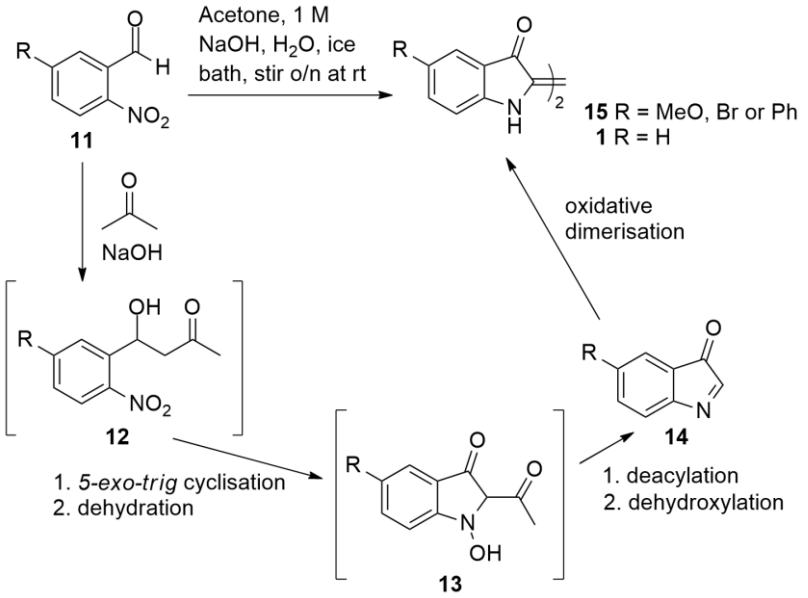
Von Baeyer received the 1905 Nobel Prize for his work between 1866 – 1883 in elucidating the molecular structure of indigo **1** but incorrectly assumed it contained a *cis* double bond. The correct *trans* configuration was first confirmed by X-ray crystallography performed by Reis and Schneider in 1928[4] and by Von Eller in 1955.[22]

The *trans* structure of indigo **1** is observed in nature because of the stabilising intra- and inter-molecular hydrogen bonding seen in Fig 3. Such hydrogen bonding interactions, in collaboration with π-stacking and Van der Waal interactions, as well as intramolecular resonance, results in extensive networks of tightly packed clusters.[4] Consequently indigo **1** is partially soluble in DMSO (dimethyl sulfoxide), DMF (dimethylformamide), nitrobenzene and concentrated sulfuric acid.[4]

Theoretical studies have shown that hydrogen bonding increases the anti-aromatic character of indigo **1** such that the lowest unoccupied molecular orbital (LUMO) and therefore absorption energy is lower in agreement with Baird aromaticity.[23,24]

Syntheses

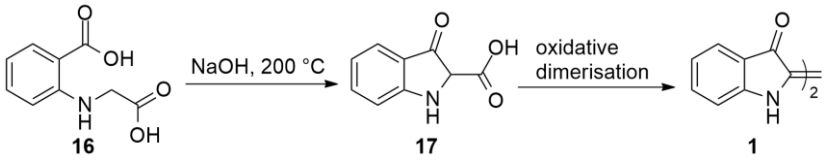
Indigo **1** was first synthesised in 1878 but it wasn′t until 1882 that Adolf Baeyer and Viggo Drewsen developed the first efficient laboratory scale method, the Baeyer-Drewson indigo **1** synthesis (Scheme 3).[16] It involved an aldol condensation of nitrobenzaldehydes **11** with acetone and sodium hydroxide to form 4-hydroxy-4-(2-nitrophenyl)butan-2-one **12** followed by a 5-*exo*-*trig* cyclisation and dehydration to give 2-carboxyl-*N*-hydroxylindoxyl **13**. This compound was deacylated and dehydroxylated to 3-indolone **14** which oxidised in air to indigo **1**.[11]



Scheme 3: The Baeyer-Drewson synthesis of indigo **1** from nitrobenzaldehydes **11** (1882).[11]

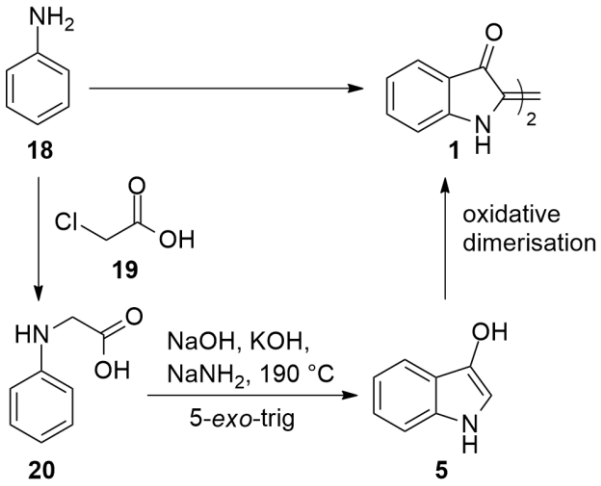
The Baeyer-Drewson synthesis was useful for obtaining indigo **1** on the laboratory scale but not the industrial scale. In 1897 Karl Heumann devised the first synthesis for the industrial scale production of indigo **1** and soon after in 1901 Johannes Pfleger devised another industrial scale synthesis of indigo **1**.[13,14]

Heumann's synthesis (Scheme 4) involved heating a mixture of 2-((carboxymethyl)amino)benzoic acid **16** and sodium hydroxide at a high temperature until cyclisation formed 2-carboxylindoxyl **17** which oxidised in air to indigo **1**.



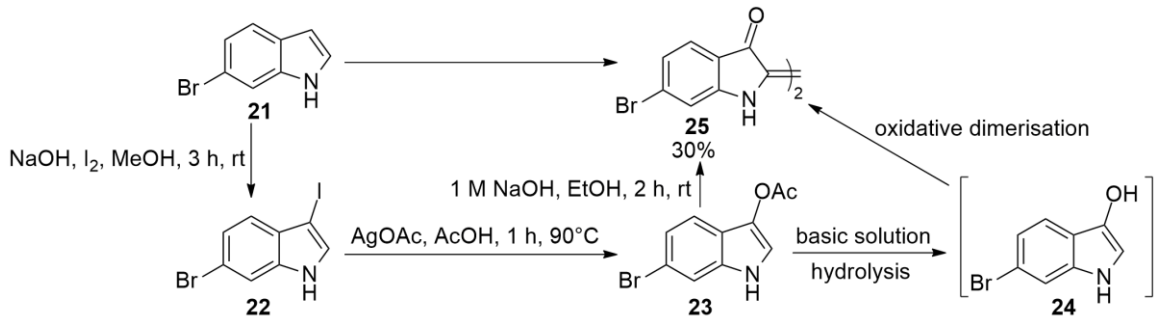
Scheme 4: Karl Heumann's industrial scale synthesis of indigo **1** (1897).[13]

Pfleger's synthesis (Scheme 5) involved reacting aniline **18** with chloroacetic acid **19** to produce *N*-phenylglycine **20** which was treated with a mixture of sodium hydroxide, potassium hydroxide and sodium amide at 190°C. This resulted in a 5-*exo*-*trig* cyclisation and subsequent dehydration to give indoxyl **5** which oxidised in air to indigo **1**.[14] Presently, over a century after its inception, Pfleger's synthesis is still the major source of manufactured indigo **1** due to the cheapness of aniline **18**.[4]



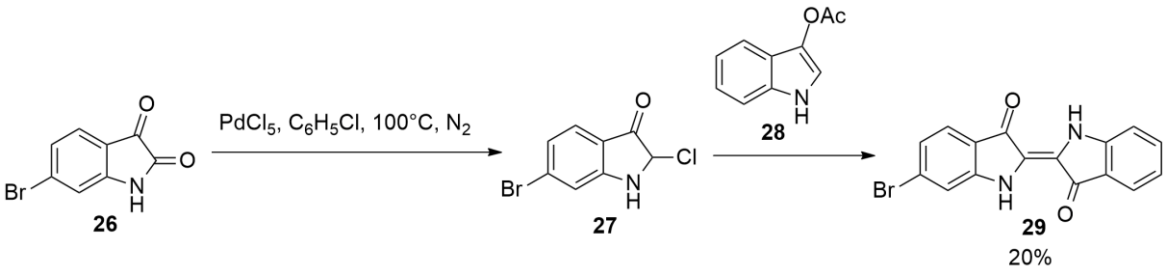
Scheme 5: Johannes Pfleger's industrial scale synthesis of indigo **1** (1901).[14]

A synthesis of 6,6′-dibromoindigo **25** devised in 2001[15] is represented in Scheme 6. 6-bromoindole **21** underwent electrophilic aromatic substitution to form 6-bromo-3-iodoindole **22** which was subsequently reacted with silver acetate to give 3-acetoxy-6-bromoindole **23**. This compound was dissolved into a basic solution wherein hydrolysis occurred to yield the 6-bromoindoxyl intermediate **24** which oxidised in air to form 6,6′-dibromoindigo **25** in 30% yield from 6-bromoindole **21**.



Scheme 6: Synthesis of 6,6'-dibromoindigo **25** in 30% yield from 6-bromoindole **21** (2001).[15]

A selective synthesis of 6-bromoindigo **29** from 6-bromoisatin **26** was reported in 1999[16] wherein 6-bromoisatin **26** was reacted with palladium pentachloride and chlorobenzene in an inert atmosphere to give 6-bromo-2-chloroisatin **27**. The addition of acetoxyindole **28** resulted in the formation of 6-bromoindigo **29** in 20% yield from 6-bromoisatin **26** (Scheme 7).[16]



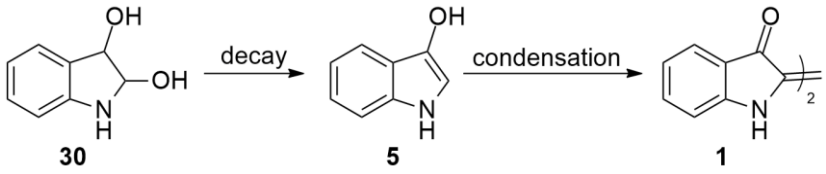
Scheme 7: A synthesis of 6-bromoindigo **29** in 20% yield from 6-bromoisatin **26** (1999).[16]

Green chemistry

Industrial indigo **1** production typically relies on the use of aniline **18** as an inexpensive fossil-fuel based material (Pleger's synthesis, Scheme 5). The success of Pleger's synthesis of indigo **1** as a viable commercial process has perhaps overshadowed the potential for environmental harm.[11] Therefore biotechnological processes are currently being explored involving the biosynthesis of indigo **1**.[4,8]

Since the 1980's a variety of redox enzymes were discovered with the ability to oxidise indole **7** into indigo **1** such as peroxygenases and indole **7** monooxygenases. Many of these indigo **1**-forming oxygenases were a part of multi-component enzyme systems but self-sufficient enzymes have also been discovered and engineered such as 2-hydroxylbiphenyl 3-monooxygenase (a flavin adenine dinucleotide (FAD)-dependent monooxygenase for which indigo **1** formation has been found). Future studies will resolve which enzyme systems are better suited to the biotechnological production of indigo **1**.[11,20,25]

The first bacterial strains capable of indigo **1** synthesis were also identified in the early 1980's. Since then most strains discovered were known as aromatic hydrocarbon-degrading bacteria. In 1983 Ensley *et al*. discovered that a system of dioxygenase-encoding genes from *Pseudomonas putida* G7, known as the multi-component naphthalene dioxygenase system (NDO), when expressed in *Escherichia coli (E. coli)* resulted in blue-coloured cultures and that indigo **1** production could be further stimulated with the addition of tryptophan **9** or indole **8** to the medium. The action of NDO in *E. coli* resulted in the production of indoline-2,3-diol **30** which decayed to form indoxyl **5** which subsequently oxidised in air to indigo **1** (Scheme 7).[26]



30 **5** **1**

Scheme 7: Indigo **1** production in *E. coli* by Ensley *et al* (1983).[21]

Since then several oxygenases, mostly from the genus *Pseudomonas sp.*, have been studied for their capacity to oxidise indole **8** to indigo **1**. Although the process of indigo **1** biosynthesis is not economically feasible due to the low solubility and high toxicity of indole **8**, biotransformation in biphasic (water-oil) systems offers the advantage of increased substrate loading as well as ensuring the aqueous phase remains untainted by toxic, lipophilic indole **8** molecules. In 2003 Doukyu *et al* used phenol hydroxylase (PH) from *Pseudomonas sp.* and its variant (OST 3410) to oxidise indole

8 to indigo **1** in biphasic systems but received poor indigo **1** production yields (12 and 52 μg/mL, respectively).[27]

In 2015 whole cells of the strain PH_IND (*E. coli* BL21 (DE3) expressing PH) were used to oxidise indole **8** to indigo **1** in a biphasic system, with dodecane making the best organic phase for this biotransformation. Optimal production of indigo **1** (243.51 mg/L) was measured in the oil phase, 2.7-fold higher than in the aqueous phase and the results of the study suggested that strain PH_IND may be an effective biocatalyst in the industrial bio-production of indigo **1**.[27,28]

It has already been demonstrated that indigo **1** and its derivatives can be selectively produced through the genetic engineering of plants despite this approach presenting competition to the agricultural industry. Nonetheless efforts were made in the late 1990s by the Indian company Delux International to once more grow and export natural indigo **1** to Europe.[4]

In 2001 the company Genencor International, Inc published a patent titled 'Microbial production of indigo **1**' which detailed the microbial synthesis of indigo **1** from glucose using a recombinant organism.[29]

Tyrian purple

Tyrian purple (TP) is an ancient indigoid dye whose use dates to 3900 YBP in Crete. See title page. TP contains indigo **1** as well as the naturally occurring reddish purple indigo **1** derivatives 6,6′-dibromoindigo **25** and 6-bromoindigo **29** (Fig 4). In antiquity (1400 – 2800 YBP) TP had a social, political and religious prestige due to its value; it was so rare and coveted that it was almost exclusively worn by kings including emperors Nero and Jusitinian.[4] It was also worn by high priests as documented in the Old Testament (Exodus 26:1, 31).[4]

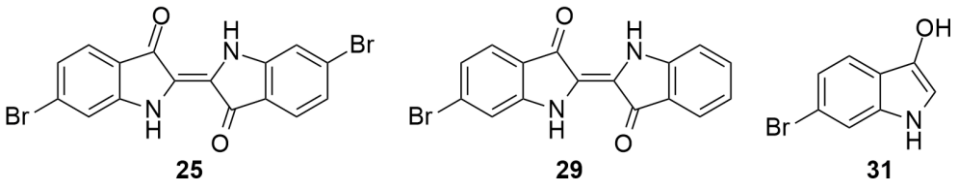


Figure 4: Two of the three dye components of Tyrian purple (6,6'-dibromoindigo **25** and 6-bromoindigo **29** excluding indigo **1** and one of two precursor compounds 6-bromoindoxyl **31** excluding indoxyl **5**.

The precursor compounds of TP (indoxyl **5** and 6-bromoindoxyl **31** (Fig 4) were found in small quantities in the hypobronchial glandular secretions of Mediterranean shellfish of the genus *Purpura* (latin for 'purple'), also known as Murex sea snails.[4] In antiquity the city of Tyre on the Phoenician coast was the centre of the TP industry, as documented in the Bible (Chronicles 2:7 and Ezekiel 27:7) and the Phoenicians established TP production facilities as far as the *Iles Purpuraires* at Mogador in Morocco.[30] The former fame of Phoenician TP is evident in the name itself; 'Phoenician' is derived from the Ancient Greek word *Phoiníkē* which means 'to make red'.[4]

When Alexander the Great (2376 – 2343 YBP) conquered Persepolis, the capital of ancient Persia, approximately 2350 YBP he found purple robes 190 years of age worth 5000 talents at the time. At about the same time (2320 YBP) Aristotle made a statement claiming that TP was valued at ten to twenty times its own weight in gold.[4] Aristotle also described the shellfish and the TP extraction process in his *History of Animals*.[13]

The first known description of the laborious extraction process of TP from Murex sea snails (*Murex brandaris* and *Murex trunculus*) was documented in *Naturalis Historia*, authored by Pliny the Elder, and involved crushing the snails, allowing them to stew in alkaline salt water for three days and then boiling them for up to ten days.[10] Later, in 1909, following this recipe Friedländer extracted 1.4 g of dye from twelve thousand molluscs and elucidated the structure of 6,6′-dibromoindigo **25**.[13]

Pliny the Elder wrote of TP that "it brightens every garment and shares with gold the glory of the triumph."[4] This statement illustrates the connection between purple dye and political power in the ancient Roman Empire. In imperial Rome, the use of TP was highly regulated and reserved for the elite. During the reign of the Roman Empire the production of TP resulted in an ecological disaster. Evidence for the occurrence of this event can be found in what used to be Sidon but is now Lebanon where there resides "Murex hill", a mound of shells wrought by past demand for TP.[13]

The TP industry died out with the decline of the Eastern Roman Empire 567 YBP, when Constantinople was conquered the by Ottoman Empire. Modern understanding of the ancient dyeing technology used by the Phoenicians is substantiated by archaeological research as well as a detailed account left by Pliny the Elder. There was still demand for prestigious purple robes 556 YBP and since no TP was available Pope Paul II's efforts to have all of the cardinals gowns dyed purple with alternative dyes resulted in their gowns appearing a progressively brilliant red.[4] TP was rediscovered 164 YBP by French zoologist Félix Henri de Lacaze-Duthiers who reportedly noticed a fisherman colouring his shirt using a shellfish.[4]

Natural products

The components of TP were the only known naturally occurring substituted indigo **1** derivatives until the 1970's when halogenated derivatives of indigo **1** were found in the New Zealand marine invertebrate *Ptychodera flava laysanica* (Fig 5).[31]

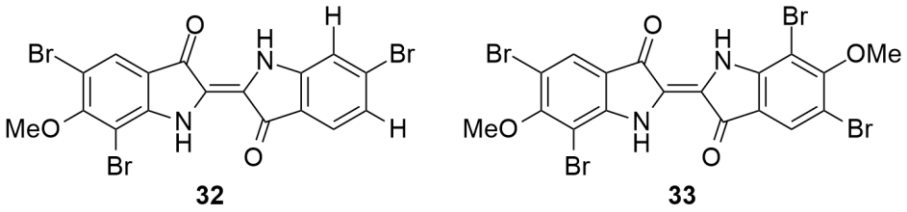


Figure 5: Halogenated indigo **1** derivatives **32** and **33**, found in *Ptychodera flava laysanica.*

Indigo **1** has also been isolated from fungi (*Schizophyllum commune* and *Agaricus campester*) and metabolic disorders such as Blue-Diaper syndrome and Purple urine bag syndrome have been correlated to the presence of indigo **1** in the urine, blood plasma and haemofiltrate of patients e.g. the illness of King George III.[16,32]

In 2002 the 5,5′-dichloro-substituted indigo **1** derivatives akashins A, B and C (**34**, **35** and **36**, respectively) were isolated from from terrestrial *Streptomyces spp* (Fig 6). These nonsymmetrical monoglycosylated compounds were previously undescribed, even as synthetic products, and exhibited significant inhibitory activities against the growth of various tumour cell lines relative to biologically inactive indigo **1**.[32] The sugar residue itself is 4-acetamido-4,6-dideoxyglucopyranose and the corresponding amino sugar is a component of over 120 pharmacologically active natural products including tallysomycin and calicheamicin.[32]

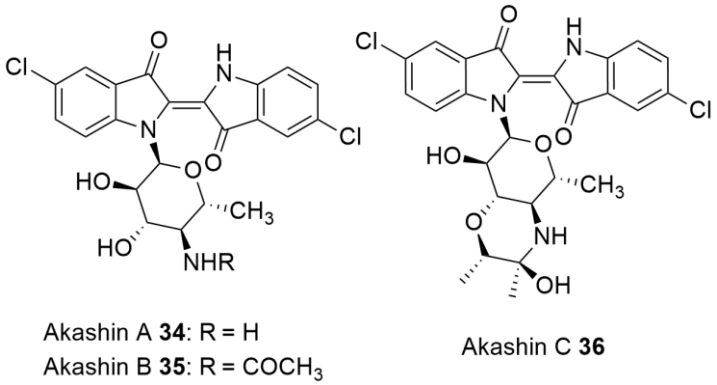


Figure 6: Akashins A **34**, B **35** and C **36**.

Natural products exist that share their 2,2′-biindoline core with indigo **1**, known as bisindole alkaloids, and some have been shown to possess medicinal value as anticancer agents, for example rebeccamycin **37**, staurosporine **38**, iheyamine A **39**, iheyamine B **40** and fascaplysin **41** (Fig 7).

The glycosylated indole **8** derivatives rebeccamycin **37** and staurosporine **38** are important anti-cancer drugs in modern clinical medicine.[33]

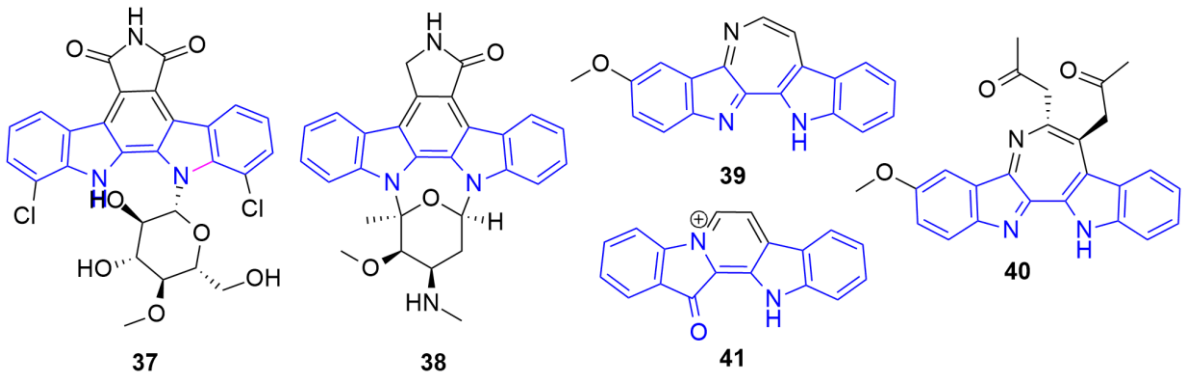


Figure 7: Rebeccamycin **37**, staurosporine **38**, iheyamine A **39**, iheyamine B **40** and fascaplysin **41**. The 2,2'-biindoline core is highlighted in blue.

The natural products seen in figure 7 were found to possess *in vivo* pharmacological activity against targets CDK4, MALME-3M melanoma cells, P388 leukemia cells and protein kinase A (PKA). The half maximal inhibitory concentration (IC_{50}) of each natural product was tested against their respective *in vivo* protein targets. Most significantly, rebeccamycin **37** showed a strong affinity (low IC_{50}) for P388 leukemia cells (IC_{50} = 1.22 μM), as did staurosporine **38** for PKA (IC_{50} = 22 nM), iheyamine A **39** for P388 leukemia cells (IC_{50} = 1 μg/mL) and fascaplysin **41** for MALME-3M melanoma cells (IC_{50} = 0.36 μM).[34]

Iheyamines A **39** and B **40** were isolated from ascidians (*Polycitorella sp.*) in 1999 on the island of Iheya in Okinawa and have shown moderate cytotoxicity against tumour cell lines.[35]

Fascaplysin **41** was first isolated from the marine sponge *Fascaplysinopsis Bergquist sp*. in 1988.[36] Fascaplysin **41** exhibited antiangiogenic and antiproliferative activities against cancer cell lines and its DNA-intercalating ability was comparable in affinity to that of clinically relevant DNA intercalators.[37] It showed strong inhibitory activity of cyclin-dependent kinase 4 (CDK4) and correspondingly prevented the growth of cancer cells during the Go/1 phase (rest/growth phases) of the cell cycle.[36] In addition **41** exhibited cytotoxic effects against chemotherapy-resistant small cell lung cancer (SCLC) cells.[36,30]

Synthetic derivatives

The indigo **1** moiety has been the subject of a series of cascade reactions, rapid syntheses which demonstrate the ability to yield a broad range of bioactive heterocycles.[38-41] Among the products yielded by the allylation of indigo **1** were representatives of the pyrazino[1,2-*a*:4,3-*a*′]diindole **42**, pyrido[1,2-*a*:3,4-*b*′]diindole **43** and benzo[*b*]indolo[1,2-*h*]naphthyridine **44** families of heterocycles (Fig 8), the latter of which contained the core skeleton of the anti-cancer and anti-plasmodial drug fascaplysin **41**.[41] Furthermore **42** contained a pyrido[1,2-*a*]indolic moiety whose derivatives have known pharmacological actions e.g. as novel non-nucleoside reverse transcriptase inhibitors of human immunodeficiency virus type 1 (HIV-1).[42]

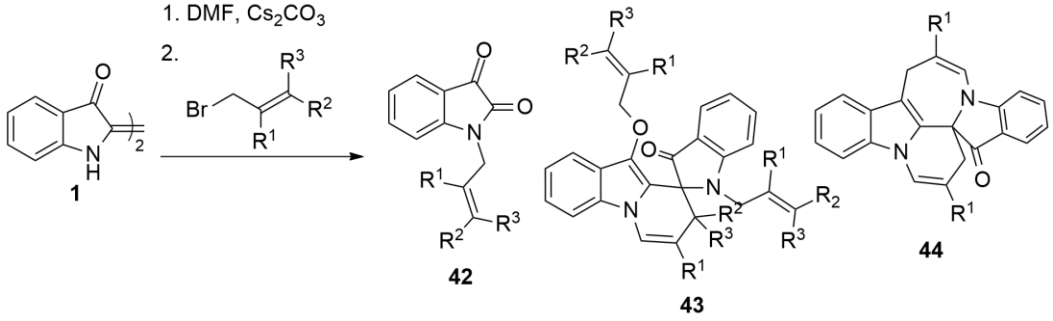


Figure 8: A cascade reaction to produce allylisatins **42**, spiroindoline–pyridoindolones **43**, and pyridoindolo-azepinoindolones **44**.

In 2013 propargylations of indigo **1** were found to yield several products with significant (micromolar) anti-plasmodial activity against a drug resistant strain including compound **45** shown in figure 9 which resembles substituted fascaplysin **41**.[39]

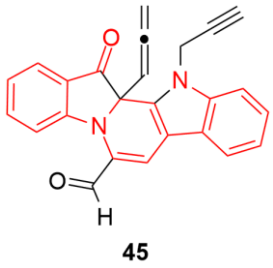
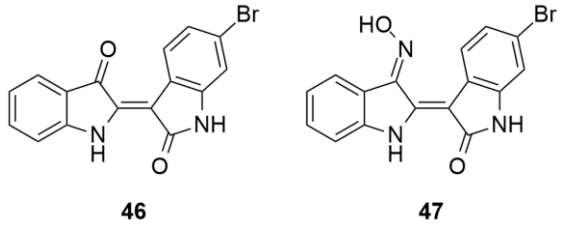


Figure 9: A bioactive propargylation product of indigo **1**, **45**. Atoms highlighted in red correspond to the structure of fascaplysin **41**.

Several glycosylated indirubin **2** derivatives also show anti-cancer activity including indirubin **2** itself. Indirubin **2** and its derivatives are known to possess a natural affinity for cyclin-dependent kinases (CDKs) and glycogen synthase kinase 3 beta (GSK-3b) giving rise to anti-tumour activity. Indirubin **2** derivatives have been developed as kinase inhibitors, targeting proteins integral in the pathophysiology of cancer. Indirubin **2** has been used in traditional Chinese Medicine for centuries to treat myelocytic leukemia among other ailments as a bioactive component of *Danggui Longhui Wan*.[43]

Recent data from X-ray crystallography of CDK2, CDK5 and GSK-3b as part of a complex with different indirubin **2** derivatives revealed information about the interactions between the functional groups of indirubin **2** and the active site resides of the kinases.[40] The two most important positions in the molecule in terms of improving the binding affinity of indirubin **2** were 3′ and 6. 6-

Bromoindirubin **46** was known to have a high selectivity for GSK-3b and the modification of the carbonyl group at the 3′ position into an oxime was performed so as to increase water solubility, cell permeability and kinase affinity. The 3′-oxime derivatives showed increased inhibitory activity toward all three protein targets *in vitro.* The compound with the highest binding affinity was 6-bromoindirubin-3′-oxime **47** (Fig 10).[40]



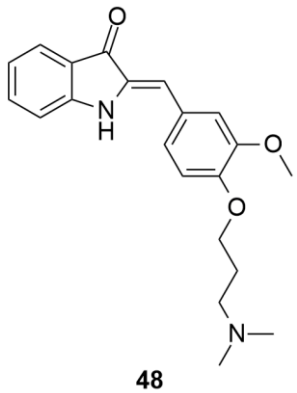
46 **47**

Figure 10: 6-bromoindirubin **46** and 6-bromoindirubin-3'-oxime **47**.

Kinases are involved in cellular signaling, indicating indirubin **2** derivatives may have other applications such as in stem cell biology to induce or maintain the pluripotency of stem cells and targeting specific proteins in the treatment of viral infections.[40]

Guengerich *et al.* (2004) found that indigo **1**, indirubin **2** and indirubin-3′-oxime **47** were potent *in vivo* ligands of the aryl hydrocarbon receptor. Indiribin-3′-oxime **47** was found by Xie *et al* in 2004 to be potent inhibitor of c-Jun NH2-terminal protein kinase (JNK), a protein involved in the regulation of neuronal apoptosis. It follows that indiribin-3′-oxime **47** may serve as an effective drug in the clinical treatment of neurogenerative disorders such as Parkinson's and Alzheimer's.[44]

In 2019 the use of hemi-indigo **48** (Fig 11) was explored as an organic photo-switch for photoinduced manipulation of nucleic acids such as RNAs which represent important targets in diagnostic medicine and medicinal chemistry.



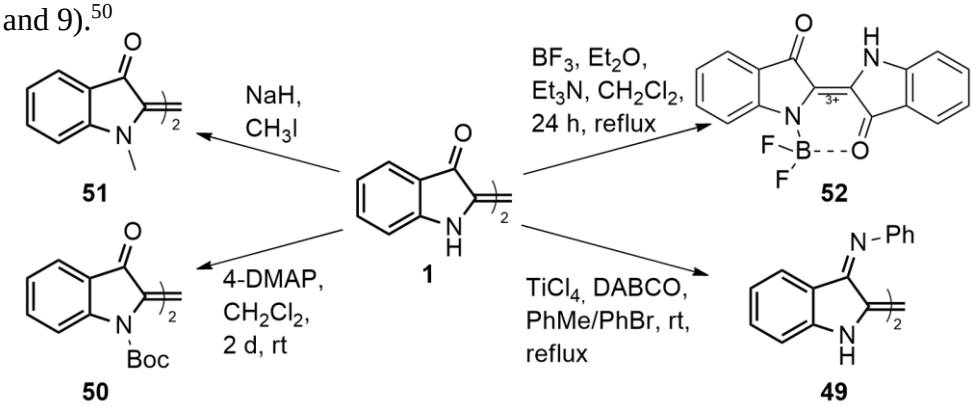
48

Figure 11: The structure of hemi-indigo **48**.

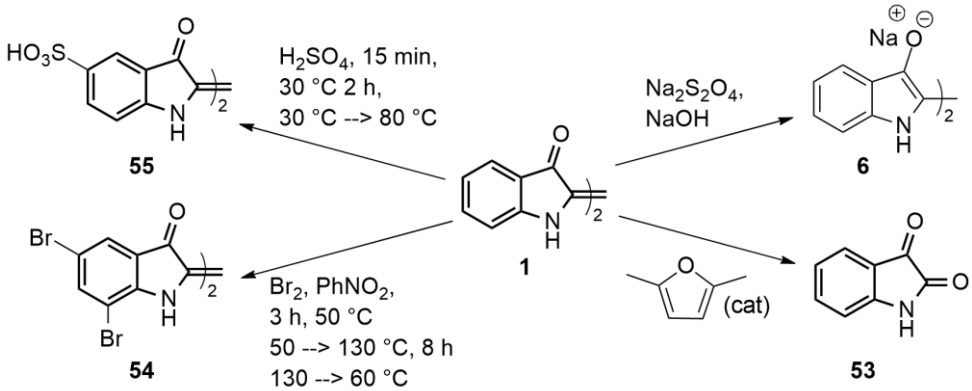
Hemi-indigo **48** was first synthesised in the early twentieth century but its photo-switching properties were formerly unexplored. The hemi-indigo **45** scaffold was found to be the foundation for a novel class of photo-switchable RNA-binders that could be switched with visible light only. These compounds were determined to be promising candidates in real-time monitoring of biological systems because the presence of a photo-switchable fluorophore allows for the implementation of novel bioimaging techniques that would otherwise be impossible.[45]

Reactions

The nucleophilic anilino group of indigo **1** has been shown to react with electrophiles in basic conditions and may for example reacted with a *tert*-butyloxycarbonyl (Boc) protecting group **50**,[47] alkylated to produce *N,N*-dimethylindigo **51**[48] or coordinated into a complex with metals such as Boron **52**.[46] Indigo **1** may also be converted to a diimine **49**.[46] Other reactions include the reduction of indigo **1** with sodium dithionite yielding leucoindigo **6** as its sodium salt,[13] oxidation in the presence of 2,5-dimethylfuran catalyst to yield isatin **53**[13] as well as 5,5′,7,7′-tetrabromination to produce Ciba blue **54**[49] (5,5′,7,7′-tetrabromoindigo) and treatment with fuming sulfuric acid to generate indigo carmine **55**,[10] a surgical dye and popular colorant of food, drugs and cosmetics (Scheme 8 and 9).[50]

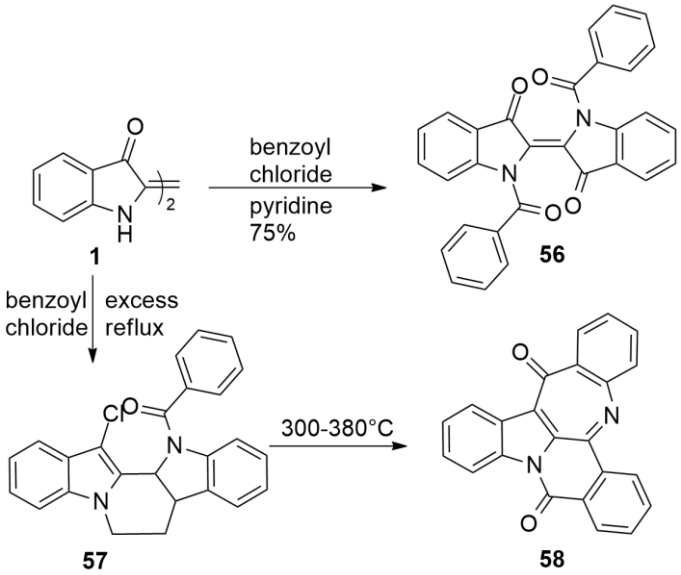


Scheme 8: Reactions with indigo **1** to yield a diimine **46**, *N,N*-dibutyloxycarbonylindigo **47**, *N,N*-dimethylindigo **48** and an indigo-Boron complex **49**.



Scheme 9: Reactions of indigo **1** to yield leucoindigo **6**, isatin **53**, Ciba blue **54** and indigo carmine **55**.

Schwartz performed the first known *N*-acylation of indigo **1** in 1863 and characterized his brown amorphous product as *N,N′*-dibenzoylindigo **56** but in 1929 the compound was properly isolated as purple crystals (scheme 10).[51] Despite Schwartz's initial failure his reaction was taken further in 1909 when Dessoulavy produced the colourless compound **57** by prolonged reflux of indigo **1** with benzoyl chloride. It was found that **57** could be further heated (300 – 380 °C) to form the dye Ciba Yellow 3G **58**, the structure of which was not elucidated until 1983 by X-ray crystallography performed by Rihs and Tzikas.[52]



Scheme 10: The reaction of indigo **1** with pyridine and benzoyl chloride to yield *N,N*-dibenzoylindigo **56** and additional reflux in benzoyl chloride to yield Dessoulavy's compound **57** which if heated at 300 - 380 °C lead to the formation of Ciba Yellow 3G **58**.

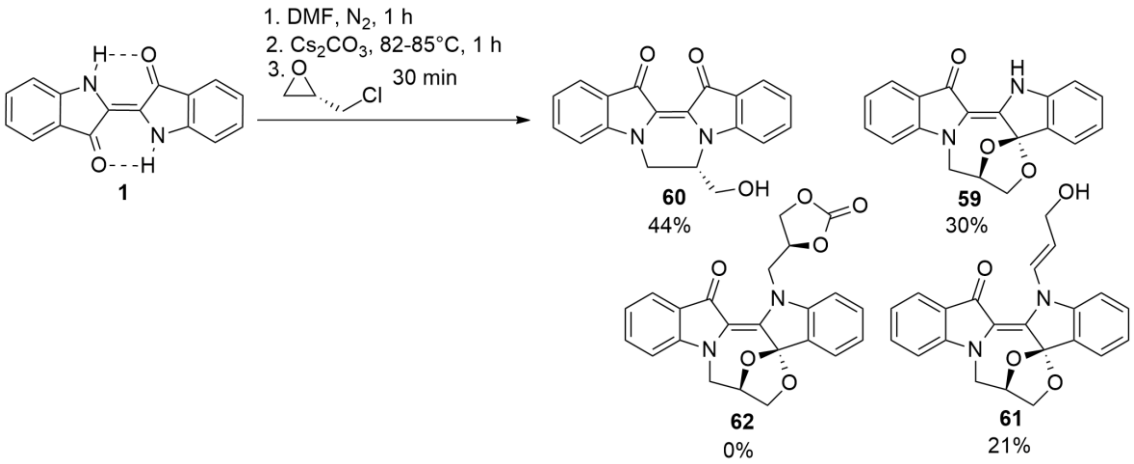
Electrophilic cascade reactions of indigo and (*S*)-epichlorohydrin

Cascade reactions are chemical processes that involve more than one consecutive reaction. It is a one-pot process and each reaction in the sequence occurs spontaneously. Multiple products are expected to be yielded from cascade reactions efficiently and economically where independent reactions to yield the same products might take much longer. The reaction can be made to select for specific products by altering the reagent, reaction time, atmosphere or temperature.[53]

Recent cascade reactions with indigo **1** have yielded promising and novel products.[32-35] One such reaction was between indigo **1** and (*S*)-epichlorohydrin. Four spiroacetal products were isolated, **59** in 30% yield from indigo **1** as small bright orange crystals in addition to products **60** and **61** (scheme 11). A fourth product **62** was found to be attainable in 6% yield by increasing the reaction time to 2 h. All four products were found to fluoresce under UV light.

Epoxide ring opening is much more favourable than direct nucleophilic halide displacement in polar solvents.[54] This arrangement allows for two successive reactions involving (*S*)-epichlorohydrin, the first being the epoxide ring opening and the second either a reformation of the epoxide (involving

the expulsion of the halide) followed by a second nucleophilic attack on the reformed epoxide or direct displacement of the halide by a second nucleophile (Appendix 1).[38]



Scheme 11: The cascade synthesis of spiroacetal products from indigo **1** and (*S*)-epichlorohydrin. The major spiroacetal products **59** was afforded in 30% yield, **60** in 44% yield, **61** in 21% yield and **62** in 0% yield.

Fluorescence

Fluorescence is a useful property in diagnostic medicine, as such compounds can be used as a non-destructive way of tracking or analysing endogenous and exogenous molecules *in vivo*; the fluorescent emission can be tracked at a specific wavelength and proteins and other endogenous molecules can also be labelled with fluorescent dyes.[55]

Examination of the photophysical properties of indigo **1** cascade product **59** in steady state experiments with yielded major absorbance bands at 295, 350 and 480 nm with extinction coefficients of 16000, 9500 and 16000 $M^{-1}.cm^{-1}$ respectively.[56] No solvatochromism was observed in the absorption features except for the 350 nm band which changed with solvent polarity. The emission spectrum (emission wavelength of 440 nm) showed fluorescence at 520 nm (this changed slightly with respect to the solvent used). Outstanding values for the fluorescence quantum yields of **59** were determined as 76, 71, 97 and 78% in toluene, THF, DCM and acetonitrile solutions respectively (Appendix 2).[56] In addition transient absorptions were measured and it was determined that only the singlet excited state (and not the triplet state) was accessed by photoexcited **59**. This observation was consistent with the quantum yields measured and it was concluded that for **59** fluorescence was the primary relaxation channel.[52]

Presently uninvestigated is the concept of introducing different electron donating (EDGs) and electron withdrawing groups (EWGs) to the diindolic system to examine how the fluorescence of the molecule is affected. The chromophore of **59** was not ascertained and therefore electronic modulation might provide necessary information.[56] Imposing steric and electronic factors on the

molecule with the addition of aromatic ring substituents to indigo **1** invariably affects its chemistry and potentially its fluorescence (Fig 12).[9]

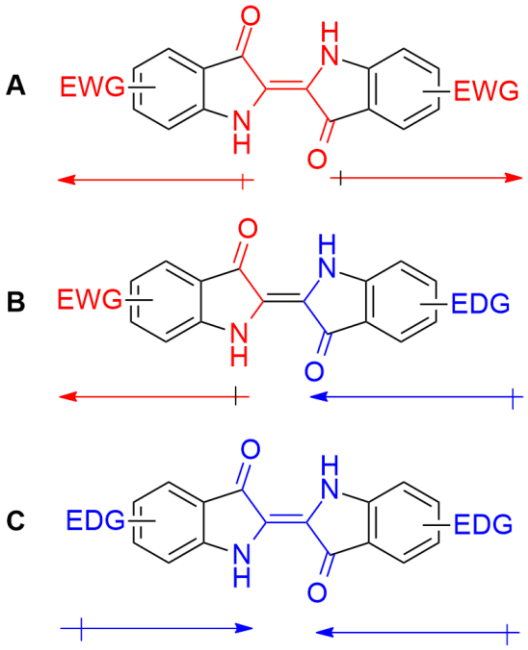


Figure 12: Varying dipoles in the indigo **1** molecule substituted with electron-withdrawing groups (EWGs) or electron-donating groups (EDGs), whose processes are coloured red and blue respectively.

Varying substituents affects the diversity of cascade reaction products by altering the reactivity of the molecule's electrophilic and nucleophilic regions and may also affect the relative proportions of each product forming. Molecule **B** in figure 12 represents the captodative effect. Put simply when the phenyl rings of indigo **1** are substituted asymmetrically with an EWG and an EDG such as illustrated in figure 13 (molecule **B**), a synergistic effect is observed leading to radical stabilisation. This is known as the captodative effect (Fig 13).[57]

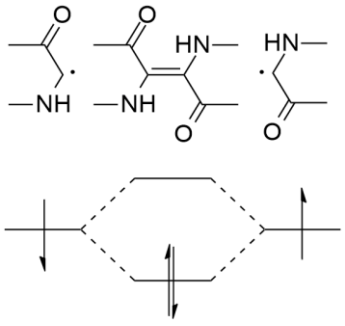
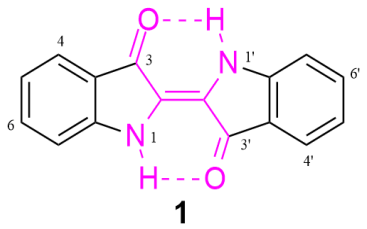


Figure 13: Ground and first excited electronic states of indigo **1** illustrating the captodaptive effect.[48]

Photophysical chemistry

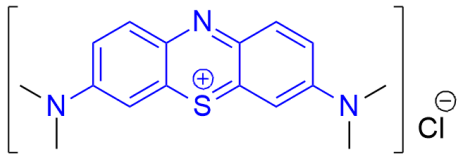
In 1876 Witt introduced the concept of the chromophore – the group of atoms in a molecule responsible for its colour – and the auxochromes, those atoms that "enhanced" the colour. It was recognised by Dilthey and Wizinger in 1928 that the chromophore tended to be an EWG while the auxochromes were commonly EDGs. The chromophore of indigo **1** is a simple cross-conjugated arrangement of two carbonyl electron acceptors and two anilino electron donors across an alkene bond (Fig 14).[58]



1

Figure 14: The structure of indigo **1** in *trans* configuration, intramolecular hydrogen bonds shown. Atoms in purple constitute the chromophore.

The chromophore of indigo **1** lacks the extensive structure that is typical for absorption of orange light (maximum absorption wavelength = 610 nm) as seen in the relative size of the chromophore of the dye methylene blue **63** (Fig 15). In accordance with these observations, indigo **1** has an excitation energy of approximately 2 eV in polar solvent, unusually low for such a compact molecule.[6]



63

Figure 15: The structure of methylene blue **63**. Atoms in blue constitute the chromophore.

In the gas phase (wherein the dye's colour is conferred by single molecules) indigo **1** is red with a maximum absorption wavelength of 540 nm whereas in solution the colour of indigo **1** is determined by the solvent in which it's dissolved (shifts to longer wavelengths) e.g. in nonpolar solvents such as chloroform indigo **1** is violet (maximum absorption wavelength of 588 nm) while in polar solvents such as DMSO it's blue (maximum absorption wavelength of 620 nm).[7] Analysis of the X-ray crystal structure of indigo **1** has shown that in its crystalline state (maximum absorption wavelength of 650 nm), molecules are tightly aggregated by intermolecular hydrogen bonding and that this is a major cause of the shift of colour compared to gas phase (monomolecular) indigo **1**.[22]

Indigo **1** hosts a rigid sp^2 bond that facilitates planarity of the molecule whereas leucoindigo **6** displays nonplanarity due to its flexible sp^3 bond.[3] The interrupted planarity of leucoindigo **6** demonstrates the importance of the chromophore of indigo **1** in producing colour.[3]

The chromophore and auxochrome theory, while useful as a simple explanation for the origin of colour in dyes, lacks rigorous theoretical justification. The blue colour of indigo **1** is better accounted for by the resonance approach as well as by molecular orbital theory.[3] Full resonance structures for indigo **1** in its first electronic excited state are found in appendix 3.[3,25]

In accordance with Planck's equation, the wavelength of maximum absorbance of a molecule increases as the difference in energy between the ground state (S_0) and the first excited state (S_1) decreases; the wavelength at which indigo absorbs is inversely proportional to the energy gap between S_0 and S_1.[25] The blue colour of indigo arises from the strong $S_1 \leftarrow S_0$ transition centred around 600 nm. In general indigo **1** absorbs at relatively long wavelengths in the visible region (600 675 nm) and the absorption of light results in excitation to a higher energy state.[3,25]

In 2004 the photophysical properties of indigo **1** and leucoindigo **6** were examined.[59] The article presented singlet and triplet excited-state studies including fluorescence, triplet absorption spectra, intersystem crossing triplet yields and triplet lifetimes. For indigo **1**, the kinetic radiationless rate constant (function of the extent of charge transfer) was one order of magnitude lower than those of leucoindigo **6**. This was explained by the low energy difference between the ground state and first excited singlet state, which allows for overlap between the vibrational levels of each state.[59]

The major deactivation pathway of indigo **1** involved internal conversion from the lowest singlet excited state to the ground state whereas the photophysics of leucoindigo **6** involved competition between internal conversion, triplet formation and fluorescence deactivation processes. Leucoindigo **6** presented more pronounced Stokes shifts of about 81 – 84 nm (difference in energy between positions of emission spectrum band maxima), suggesting an excited state geometry different to that of the ground state, not involving the intramolecular hydrogen bonding seen with indigo **1**, which had a much smaller Stokes shift of around 40 nm, but possibly involving rotational photoisomerization across the C=C bond. The high Stokes shift observed for leucoindigo **6** may imply geometrical freedom relative to the rigid structure of indigo **1**, suggesting leucoindigo **6** may lack the photostability of indigo **1**.[60]

Usually a double bond undergoes *trans-cis* isomerisation more readily when part of a conjugated system but indigo **1** is an exception to this rule. Indigo **1** did not isomerise from *trans* to *cis* configuration (Fig 16) even when photoexcited, nor did it do so through first forming its keto-enol tautomer; only leucoindigo **6** isomerised. The resistance of indigo **1** to photoisomerisation is essential to its photostability.[62]

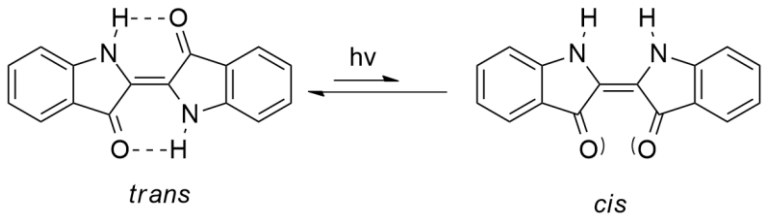


Figure 16: Equilibrium reaction between the *trans* and *cis* isomers of indigo in response to light. Light tends to prefer the stablised and conjugated structure of the *trans* isomer.

Excited-state proton transfer (ESPT) (otherwise known as protoisomerisation) was supposed to play a role in the photostability of indigo **1** but in 2016 the *cis-trans* isomerisation mechanisms of indigo **1** and its monoimine and diimine derivatives were investigated using computational chemistry and it was determined ESPT was not a factor in the photostability of indigo **1**.[61]

It has been a matter of debate whether indigo **1** undergoes ESPT. In 2015 it was argued that indigo does not undergo ESPT due to the kinetics of its transition state; supposedly the orientations of the OH and NH bond formed in protonation point away from each other to avoid steric hindrance, resulting in a high energy transition state (Fig 17). The central alkene bond also corresponds to the unbonded singlet diradical structure at the transition state (Fig 14).[3,57,63]

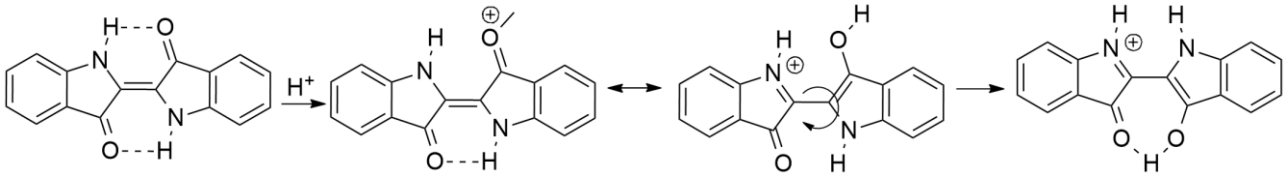


Figure 17: Protonation of one of two identical carbonyl oxygen atoms in indigo **1** allowing for a single hydrogen bond to connect the two subunits. *Trans* to *cis* isomerisation of protonated indigo **1**.[55]

In the excited state (S_1), indigo **1** has a high photo-stability and distinct deactivation mechanism which has been the subject of much discussion involving theories of fast intramolecular proton transfer and internal conversion. In 2009 a transition state was identified in photoexcited indigo carmine by observing changes in IR spectra peaks.[64] The study concluded with direct evidence that photoexcitation of indigo carmine **55** to S_1 results in the initiation of a stepwise proton-transfer mechanism (ESPT), and that the reaction which sees the molecule revert to S_o takes only 0.5 ps.[64]

Recent data[62] has confirmed ESPT does occur for indigo **1**. The barrier to isomerisation is approximately 125 kJ/mol (whereas neutral *trans*-indigo **1** is stabilised by 68 kJ/mol due to its hydrogen bonds) but *trans* to *cis* isomerisation may occur by virtue of protonation.[62] The carbonyl oxygen atom is the preferred site of protonation, whereupon the charge is delocalised throughout the conjugated system as seen in figure 17. A proton bridge may form between the protonated oxygen atom and the second oxygen atom, stabilising the *cis* structure. The *trans* configuration is destabilised upon protonation as the new partial positive charge on the protonated carbonyl oxygen destroys the carbonyl-anilino hydrogen bond strength. Computational studies indicate that the protonated *cis*-indigo **1** is lower in energy than *trans*-indigo **1**.[62]

In 2007 a singlet to triplet state energy splitting of (0.91 +/- 0.10) eV was observed for indigo **1**, taking singlet energy as an intersection of the absorption and fluorescence spectra of indigo **1** (635 nm, 1.95 eV), and, using data from pulse radiolysis experiments, a rate of intersystem crossing of 4.7×10^7 s^{-1} and noted these values were similar to those expected of aromatic hydrocarbons.[55] This

provides the important information that the lowest excited singlet and triplet states (Fig 18) of indigo **1** must be of π, π* origin.[65]

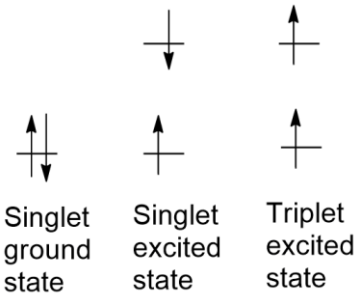


Figure 18: Possible spin states for ground and excited state molecular systems.

In 2015 geometry optimisations of indigo **1** and its derivatives in both ground and excited states were performed *in vacuo* using computational quantum modelling methods.[66] These calculations showed that indigo **1** in ground state (S_0) possessed carbonyl group bond lengths of 1.227 Å, anilino group bond lengths of 1.010 Å and intramolecular hydrogen bond lengths of 2.284 Å. When substituted with halogens on its aromatic ring, the planarity was found to be conserved, while carbonyl and anilino group bond lengths changed little. When photoexcited to S_1 one intramolecular hydrogen bond lengthened to 2.474 Å and the other shortened to 2.080 Å. This was consistent with infrared (IR) spectral shifts observed.[66] Upon photoexcitation, the dihedral angle of indigo changed by 0.014^o, thus maintaining its planarity.[66]

Halogen substituents on the aromatic ring of indigo **1** scarcely affect the intramolecular hydrogen bonds, whereas the change of electron densities in the carbonyl and anilino groups have a significant effect on intramolecular hydrogen bonds. The hydrogen bond is closely related to the excitation energy (E_A) of a molecule. Charge distribution and molecular orbital theory may be of use in understanding this phenomenon.[64] The S_1 state of indigo **1** involves the orbital transition from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO).[56] Upon excitation a charge transfer occurs, primarily from the electron donor anilino group to the electron acceptor carbonyl group. The electron density of the anilino groups decreases sharply from HOMO to LUMO while the electron densities of the carbonyl groups increases; the electron density of the central alkene bond decreases while that of the five-membered ring increases (Fig 19).[66] The electron densities of the anilino groups of indigo **1** decrease drastically from HOMO to LUMO whereas those of the carbonyl groups differ only a little. The electron density on the central alkene decreases and that of the five-membered ring increases following the transition from HOMO to LUMO (Fig 19).

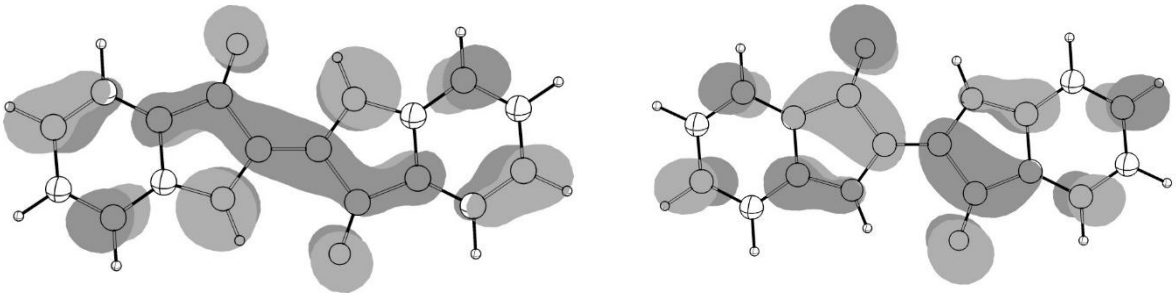


Figure 19: Illustration of the highest occupied molecular orbital (HOMO) (left) and the lowest unoccupied molecular orbital (LUMO) (right) of indigo **1**.[47]

In 2004 it was found that substitutions at different ring positions of indigo **1** were likely to promote both long and short visible and UV wavelength spectral shifts.[60] In indigo **1** derivatives 6,6′-dibromoindigo **25** and 5,5′-dibromoindigo **64** (Appendix 4) the electron density was shown to be localised on the halogen groups in HOMO and decrease almost entirely in LUMO.[64] This was taken further in 2015 when it was claimed that the site of substitution, and not the substituent itself, that was the main influence on the spectral properties of the derivatives of indigo **1**.[66] Both 5,5′- and 6,6′-disubstituted indigo **1** caused the UV spectra to redshift slightly; the absorption spectrum of indigo **1** was 535 nm whereas 6,6′-dibromoindigo **25** and 5,5′-dibromoindigo **64** were 541 nm and 545 nm respectively. Furthermore 4,4′-substitutions decreased the E_A of S_1 and caused a spectral redshift to 595 nm and *N,N*′-diacetylindigo **65** (Appendix 4) was significantly red shifted to 610 nm. However, the latter was probably due to the absence of hydrogen bonding. The difference between values of absorption and emission spectra was only about 10 nm for indigo **1** and its derivatives and the energy loss was sufficiently small to be negligible.[66]

Conclusion

Indigo **1** has a rich historical background as a dye and a pigment of ancient cultures. The story of TP is similarly interesting and culminated in an ecological disaster due to its demand being so great. Indigo **1** held great value as an agricultural crop until synthetic chemistry inherited the industry in the early twentieth century. In modern organic chemistry indigo **1** represents a cheap organic scaffold with discrete nucleophilic and electrophilic regions labile by many different reactions.[34]

The use of fossil fuel based aniline **18** in Pfleger's synthesis of indigo **1** is unsustainable. Recently indigo–forming enzyme systems were discovered and future studies will resolve which are better suited to the biotechnological production of indigo **1**.[56]

Indigo **1** is structurally unique dye in that it absorbs at long wavelengths for such a small molecule.[3,4] The indigo **1** molecule is symmetrical belonging to the C_{2h} point group. Indigo **1** and its derivatives are low HOMO-LUMO gap donor-acceptor derivatives.[21] The transition from HOMO to LUMO is associated with excitation and the formation of a charge-transfer diradical state (Fig 13).

Triplet state energy splitting and rate of intersystem crossing values of indigo **1** are like those of aromatic hydrocarbons. This provides the important information that the lowest excited singlet and triplet states (Fig 18) of indigo **1** must be of π, π^* character.[65]

The mechanism by which indigo **1** relaxes from an excited state to ground state has been a matter of debate in the past few decades. However recent data has conclusively determined this mechanism to be ESPT (Fig 17).[62] In its excited state indigo **1** changes electronically and sterically (Appendix 3). The site of substitution and not the substituent itself was determined to be the main influence on the spectral properties of indigo **1** and its derivatives.[63]

In recent times there has been a resurrection of indigo **1** based chemistry, leading to the development of cascade reactions and cascade products with unique photophysical and medicinal properties,[38] providing a rich source of inspiration for new families of π-conjugated molecular systems.[21] Some cascade products revealed outstanding quantum yields and others significant affinities for *in vivo* disease targets. The 2,2′-biindoline core represents an attractive lead compound in drug discovery. There is enormous potential for indigo **1** and its derivatives as semiconductors, candidates in medicinal chemistry and in fluorescence-based applications such as in microscopy or diagnostic medicine.[32-25] This could lead to practical outcomes and therefore it is imperative to further develop cascade reactions and to test the products for medicinal and photophysical properties.

Abbreviations

YBP – years before present

UV – ultraviolet

DMSO – dimethyl sulfoxide

DMF – dimethylformamide

LUMO – lowest unoccupied molecular orbital

HOMO – highest unoccupied molecular orbital

FAD – flavin adenine dinucleotide

NDO – multi-component naphthalene dioxygenase system

E. coli – *Escherichia coli*

PH – phenol hydroxylase

TP – Tyrian Purple

CDK – Cyclin-dependent kinase

Go/1 phase – rest/growth phases of the cell cycle

SCLC cells – small cell lung cancer cells.

PKA – protein kinase A

IC_{50} – half maximal inhibitory concentration

HIV-1 – human immunodeficiency virus type 1

GSK-3b – glycogen synthase kinase 3 beta

JNK – c-Jun NH2-terminal protein kinase

Boc – *tert*-Butyloxycarbonyl protecting group

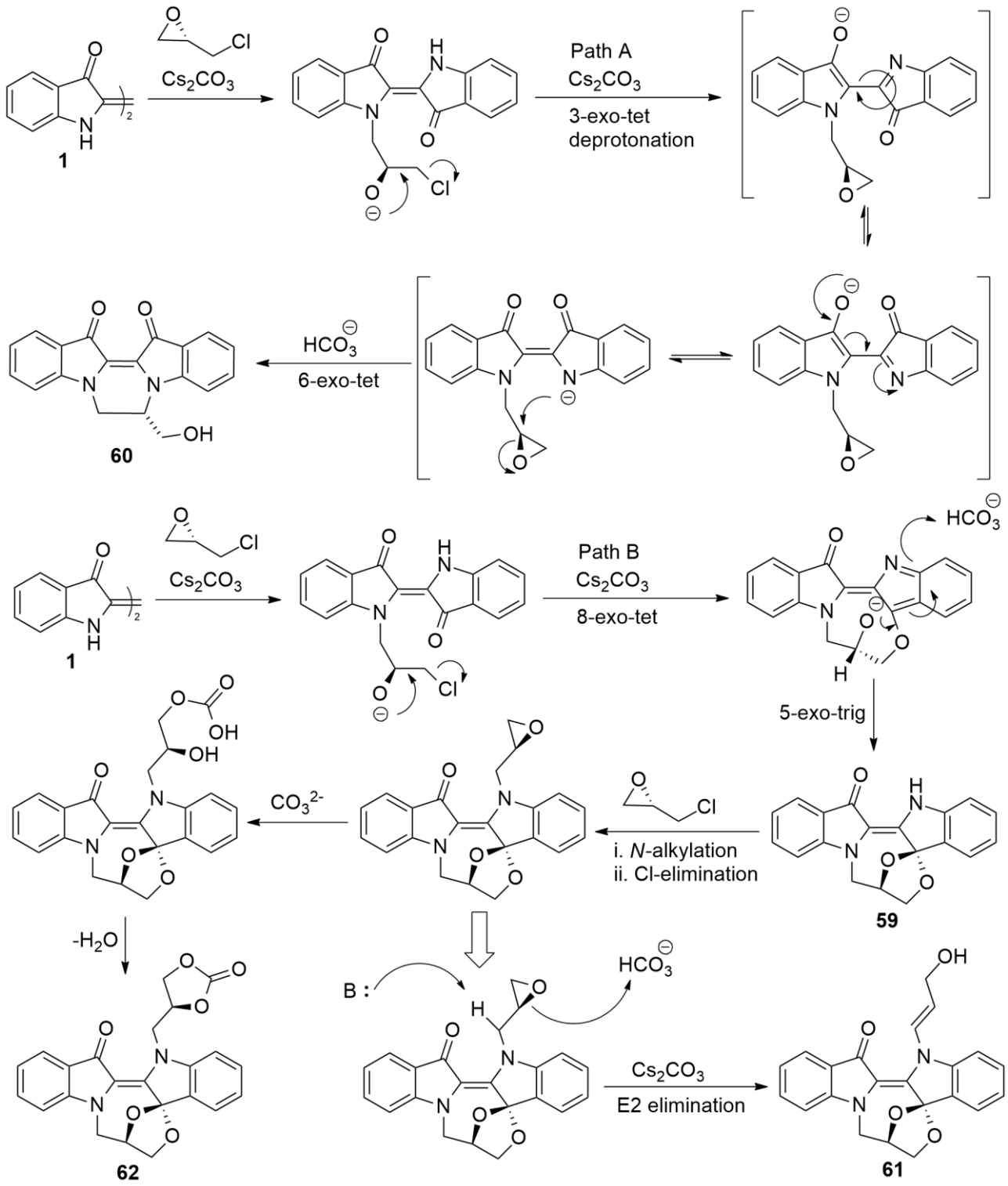
EDG – electron donating group

EWG – electron withdrawing group

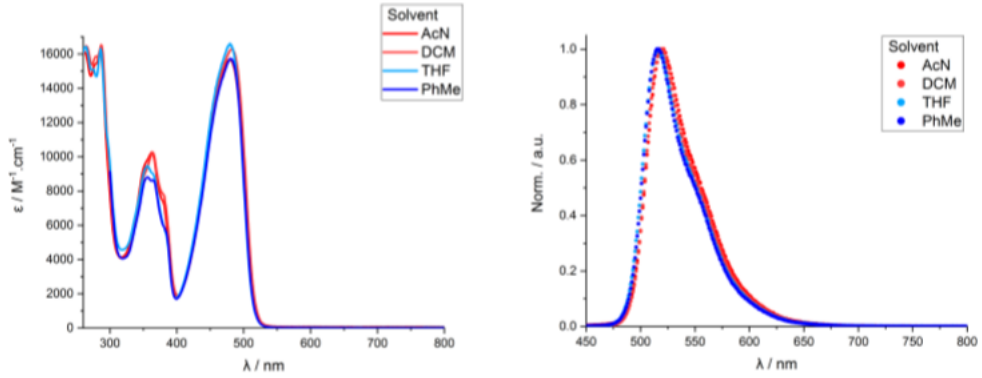
E_A – excitation energy

ESPT – excited-state proton transfer

Appendix

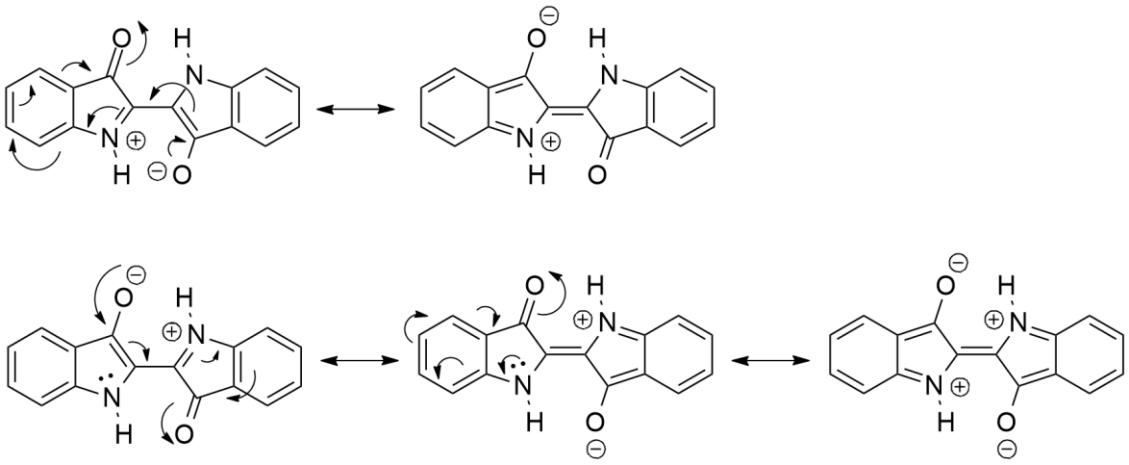


Appendix 1: Proposed mechanisms of formation of the spiroacetal cascade products.[32]

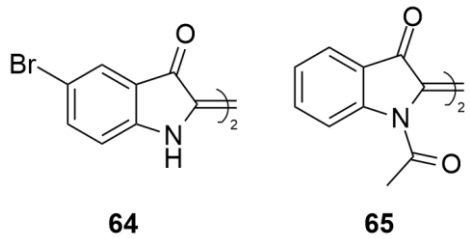


	PhMe	THF	DCM	AcN
Φ_F	76%	71%	97%	78%

Appendix 2: Steady state absorption and emission spectra for **59** dissolved in solvents of varying polarity. Excitation at 440 nm for emission spectra and fluorescence quantum yield, which are given as percentage averages from a total of four independent measurements. Tested against **59** in ethanol as the reference.[50]



Appendix 3: Resonance structures of the first electronic excited state of indigo **1**.[3]



64 **65**

Appendix 4: The molecular structures of 5,5′-dibromoindigo **64** and *N*,*N*′-diacetylindigo **65**.

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